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High-throughput method for the analysis of sterols in food samples by gas chromatography without previous fractionation steps

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Sterols are characterized by the presence of four rings and an alcohol group. Cholesterol (CHO) is the most common found sterol in animal sources while -sitosterol, campesterol, and estigmasterol are in plants and ergosterol in fungi [1]. They have attracted much attention in the last years, since CHO has been associated to cardiovascular diseases, while phytoesterols have shown anti-inflammatory properties [2]. The analysis of these compounds is quite complicated as they have low volatility and solid phase extraction purification steps are required. This research works aims to develop an analysis method by GC avoiding fractionation steps.

Cooked tuna (CT) and commercial canned tuna (NT), fish (FO), soya (SY) and krill (KR) oils were assayed as follows: lipids were isolated according to Matyash *et al.* [3]; afterwards 5β-cholestan-3α-ol was added to samples (internal standard) and all fatty acids (free and/or esterified) were converted into fatty acyl methyl esters (FAME) [4]. Finally, the sterol fraction was derivatized into trimethylsilyl derivatives (TMS) using bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) [5]. Identification was carried out by injection of pure sterol standards.

Preliminary trials showed presence of interference peaks not corresponding to sterol compounds. However, when derivatization was carried out using only glassware such peaks did not appear in the chromatogram. Determination of linearity, recovery and precision showed satisfactory values according to previously recommended acceptance criteria [6]. FAME derivatization allowed to eliminate any interference for lipids eluting in the sterol region. The obtained data showed that sterol fraction from CT and FO, was only composed of CHO while SY and NT had also campesterol, stigmasterol and -sitosterol. In KR samples, besides CHO, desmosterol was detected.

The proposed method allowed reliable analysis of sterols by GC as TMS in food samples bypassing the need of fractionation steps by using FAME derivatization.

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